OPSONIC INDEX AND VACCINE THERAPY

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It is the appearance of a newly coined word which often sheds a halo of mystery about a comparatively simple subject. Such a mystery seems to radiate from the term "opsonin," and it is our desire that all nurses shall understand both the meaning and the value of opsonic therapy, which is arousing such interest in the present day professional world.

The term "opsonic" is a Greek word meaning "I prepare food for," and opsonins are substances whose presence has been demonstrated in the blood of both man and the lower animals, the function of such substances being to prepare bacteria as food for the white blood corpuscles.

The terms toxins and antitoxins are no longer vague to you because you know how they work; they belong to your everyday vocabulary, and such shall be the case with opsonins as soon as you understand their function.

Bearing in mind the mechanism of immunity, which consists in raising the resistance of an individual against a given microörganism, you are master of the fundamental principles of opsonic therapy, which is nothing more nor less than the process of immunizing the individual against invading bacteria. You treat a diphtheria child with antitoxin derived from the blood serum of a horse which has been immunized against the bacillus diphtheriæ, or vaccinate a man exposed to small-pox, by introducing into his lymph channels, the lymph obtained from a cow with cowpox. These things you do to create in the blood of the individuals such an excess of antibodies to combat the invading germ and its toxins, that the individual in question shall become immune to the given microörganism.

When pathogenic bacteria gain entrance to the body, the outcome depends upon two factors: (1) The infecting agent, and (2) the individual infected.

(1) The infecting agent owes its morbid success to three main things: (*a*) Its virulence, or power to multiply in the body and cause disease, (*b*) the number of bacteria which are introduced, for we know

that tissue whose resistance has not been lowered, can withstand a certain number of pathogenic bacteria, (*c*) the pathway of infection, *i.e.*, whether the organisms enter the blood stream directly, as in septicemia, or are localized, as in an abscess.

(2) The individual infected owes his resistance to four main protective powers of the blood, which combat the invading bacteria and their toxins. These four protective agencies are in character: (1) bactericidal, or having the power to kill bacteria; (2) bacteriolytic, which includes not only the power to kill but to dissolve bacteria; (3) agglu-

FIG. 1.

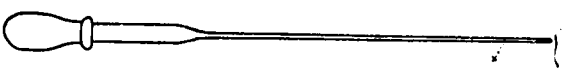
The completed capsule. Note the fine stabbing-point at the straight end *x*. From D. J. C. Hollister's paper, Jour., Surg., Gyn., Obs., Vol. III, No. 6, 1906.

tinating, or possessing the power to produce clumping of bacteria; (4) phagocytic, or the power of leucocytes to engulf and digest bacteria.

One or all of these may act on one class of microörganisms, but all four protective powers do not necessarily act the same way on all bacteria. For example, diphtheria antitoxin has given such marvellous results that we no longer dread the sound of the term diphtheria. However, serum has been prepared in exactly the same way to combat many other diseases, with but very little success.

It is to the phagocytic power of the white blood corpuscles that

FIG. 2.



The finished pipette, with rubber teat applied and a volume marked off by blue pencil (x). From Dr. J. C. Hollister's paper, Jour., Surg., Gyn., Obs., Vol. III, No. 6, 1906.

Wright and Douglas and their followers have so successfully looked for aid to fight the morbid progress of several different classes of pathogenic organisms discussed further on. They have found it possible to artificially increase the production of opsonins in the blood by subcutaneously injecting into the patient a carefully measured quantity of vaccine (sterile bacteria). The increased phagocytosis which results, is not due to any direct stimulation of the leucocytes. The newly formed opsonins are in the blood serum and act in some unknown way on the bacteria, so changing them that the white blood corpuscles, or scavengers of the body, greedily eat them up. Careful experiments have shown that leucocytes

washed free of serum and brought into contact with an emulsion of bacteria, show no phagocytic action, while if they are brought into contact with the same organisms which have been previously bathed in blood serum and the serum then carefully washed off, the microbes are rapidly engulphed by the leucocytes.

A personal vaccine is prepared when possible, by isolating the organism from the infected individual, but as the preparation of the vaccine requires several days after the organism has been isolated, the first dose is usually given from the "stock bottle." Stock bottles of vaccine made from pure cultures of various kinds of bacteria are kept in an opsonic laboratory, each bottle of course containing only one kind of bacteria, e.g., staphylococcus. Since it requires nearly three months to cultivate the tubercle bacillus on artificial media, it is plainly evident that it is impracticable to treat tuberculous patients with personal vaccines.

The normal opsonic index, as discussed below, is 1.0, that is, there is in the blood of normal individuals practically an equal measure of opsonins. The object of opsonic therapy is to raise and maintain the opsonic index as high as possible above normal, for as long a period as possible, thus constantly preparing multiplying bacteria as palatable food for the leucocytes, until the infected individual has become immunized against the infecting organism.

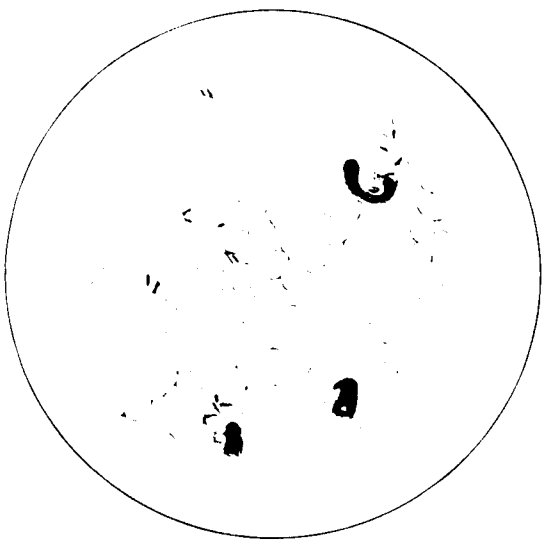
The blood is examined on the first two or three days to see whether or not there be a negative phase and to determine the maximal rise of the opsonic index. The negative phase consists in the index falling lower than it was when the vaccine was given, and has occurred but rarely in our experience. On the sixth or seventh day the index is again determined and as soon as it falls to one or below, another injection is given. Although there are many chances for error in the technique, we sincerely feel that the opsonic index is a valuable guide in regulating the time and size of the dosage, and should be carefully followed until we find a better guide.

TECHNIQUE

To measure the amount of opsonins in the blood, we must measure the degree of phagocytosis. In other words, we must determine the opsonic index. There are three main elements necessary to determine the opsonic index, *i.e.*, leucocytes, blood serum, and bacteria.

1. Leucocytes: Ten to fifteen drops of blood are dropped into a solution of sodium citrate in physiological salt solution, gently mixed and then centrifuged five minutes. The leucocytes are thus washed free of serum and are to be found in the upper layer of corpuscles after the supernatant salt solution is drawn off.

Fig. 3.



Leucocytes containing tubercle bacilli under oil immersion. From Dr. J. C. Hollister's paper, Jour., Surg., Gyn., Obs., Vol. III, No. 6, 1906.

2. Blood serum: A few drops of blood are drawn into a glass capsule (Fig. 1), the capsule sealed and centrifuged so that the blood corpuscles are thrown to the bottom, leaving the clear serum on top.

3. Bacteria: The bacteria which are causing the disease to be treated are grown on agar for twenty-four hours, the growth scraped into physiological salt solution to form an emulsion, mixed thoroughly and diluted so that there will be about one to two germs to be ingested by each leucocyte.

Equal volumes of leucocytes, blood serum, and bacterial emulsion are drawn into a capillary pipette (Fig. 2) and mixed by blowing back and forth on a glass plate. The end of the pipette is sealed and the whole incubated for fifteen minutes. The contents are then mixed as before, spread on slides and stained (Fig. 3). The number of bacteria ingested by fifty leucocytes is divided by the number obtained when the blood serum of a normal person is used in place of the blood serum from the patient and the quotient is the opsonic index of the patient.

BACTERIAL VACCINES

Bacterial vaccines are emulsions of a known number or weight of dead bacteria in physiological salt solution.

The bacteria are grown in large quantities on agar and the growth washed off with salt solution into large glass tubes which are then sealed. The clumps of bacteria are broken up by shaking the emulsion vigorously for an hour. The number of bacteria per c.c. is then counted. There are sometimes as many as seven thousand or eight thousand million bacteria per c.c., so that the counting is done by diluting a minute but definite amount of the emulsion with a known amount of salt solution, and comparing the number of bacteria in this diluted portion with the same volume of blood which we know contains five million blood corpuscles per c.c. The original emulsion is diluted with salt solution to a convenient strength for administration. The vaccine after being kept at 60° C. for an hour to kill the bacteria, is stored in brown bottles with rubber surgeon's finger tips fastened over the mouths of the bottles. Every step in the preparation must be taken with great care so as not to contaminate the vaccine. Before administering it, however, some of it is withdrawn from the bottle and planted on agar to see whether it is sterile. If no growth appears the vaccine is pronounced sterile and will keep indefinitely. To give a dose of vaccine the rubber top is dipped in lysol, the bottle held inverted, the rubber top pierced by a sterile hypodermic needle and the required amount of vaccine withdrawn into the hypodermic syringe, and injected subcutaneously. The tubercle vaccine is prepared by weight from Koch's new tuberculin.

Vaccine therapy has not been used in all infections; there are certain classes in which it has proven itself to be especially applicable. There has been perhaps the most rapid and marked results thus far in infections due to the pus forming microörganisms, *e.g.*, staphylococcus, streptococcus, B. pyocyaneus and, B. coli communis, in such diseases as Forunculosis, Acne, Abscess formation, etc. On needs but to see Acne, a disease so persistently unyielding to the usual local and systemic treatment, or Forunculosis, of years' standing and constantly recurring foruncles in spite of all treatment, yield rapidly and surely to vaccine inoculations, to realize the great value of vaccine therapy.

Tuberculosis presents an extensive field for vaccine treatment and results here have been gratifying in materially shortening the course of the disease, and instituting cure where other forms of treatment failed. This is more especially true of localized Tuberculosis such as bone and joint Tuberculosis, Tubercular Adenitis, Lupus, Tuberculosis of the Genito-urinary tract. Much preliminary investigation remains to be done before any conclusions can be drawn as to the value of vaccine treatment in pulmonary Tuberculosis.

In Gonorrhea, we are not in a position to draw clinical conclusions, although several physicians have reported very striking results in chronic cases. In our laboratory, some thirty-five cases of Gonorrhea have received vaccine inoculations. It was difficult to keep track of these cases, many of them leaving the hospital without warning. All were improved and some cured, but we were able to use the vaccine treatment alone in only three cases, the others receiving the regular routine treatment for Gonorrhea. Two of these cases showed prompt and rapid improvement, the third shows very little improvement as yet.

Certain general principles of treatment can be learned by a study of a large number of cases.

1. A dose of vaccine causes a rise in the opsonic index usually on the second or third day, followed by a gradual drop until it reaches 1.0 or below. There may be many variations from this.

2. Vaccine should be given when the opsonic index remains stationery about or below the normal line. This commonly occurs between the seventh and tenth days, thought it may not occur for two weeks or more.

3. The maximum rise of the opsonic index, the time when this occurs, and the length of time the index remains above normal are the guide marks in determining properly sized and spaced doses of vaccine.

4. The average dose of Tubercle Vaccine is 1/1000 mg. of tubercle bacilli, of Staphylococcus Vaccine 500 to 1000 million staphylococci of Gonococcus 5 to 10 million gonococci.